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Is there a common mechanism for the progression of different types of renal diseases other than proteinuria? Towards the unifying theme of chronic hypoxia

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Is there a common mechanism for the progression of different types of renal diseases other than proteinuria? Towards the unifying theme of chronic hypoxia. The question of why chronic renal diseases progress is a topic only recently investigated. Putative causes such as proteinuria do not account for all aspects of progressive renal disease. An alternative mechanism, chronic hypoxia, is proposed that might better explain certain elements of progressive renal disease, but elements of the hypothesis remain subject to further study.

Within recent memory, enthusiasms and firmly held beliefs have come and gone with regard to the question of why most chronic renal diseases progress. The need for embracing this area as being of prime importance to nephrologists is largely by default, since, if we cannot prevent disease (because we do not know what causes it), the next best option is to limit it as far as possible. Interestingly, disease progression in nephrology was a virtually untouched area until about 15 years ago when it surfaced in a big way. The discovery by Brenner and colleagues that alterations in glomerular hemodynamics could account for disease progression in the rat, precipitated a flood of studies in animals and in man. It emerged that a concept that could be understood by all and which could be pursued in simple experiments would lead to a search not only for mechanisms, but for therapies relevant to the purported mechanism. At a relatively early stage, however, we pointed out that rats are not humans and that bets needed to be hedged with regard to the applicability of glomerular adaptations in rodents to disease progression in man [1]. One reason for this was the fact that in other species, such as dogs and rabbits, renal diseases do not mimic the disease in rats. Another reason related to the interesting observation that in renal biopsy specimens from patients with a variety of diseases, the degree of tubulointerstitial fibrosis was more closely correlated with the reduction in glomerular filtration rate (GFR) than was glomerular pathology, suggesting that glomerular injury was not the major determinant of disease progression [2, 3]. There followed a period where the process of nephron hypertrophy was regarded as being relevant to glomerular fibrosis and loss of function [4]. However, cause-and-effect relationships have never been established.

Key words: progressive renal disease, proteinuria, hypoxia, fibrosis, tubulointerstitium

PROTEINURIA AS THE CAUSE OF TUBULOINTERSTITIAL SCARRING: UNANSWERED QUESTIONS

Attention subsequently has been directed to the tubulointerstitium; in this context, we are now in the throes of a new phase of enthusiasm pointing to proteinuria as the supposed culprit in renal scarring. The idea is that protein, which leaks through the diseased glomerulus, injures the tubular cells and thereby involves the interstitial compartment through the mediation of cytokines and infiltrating cells [5]. This idea is supported by a number of in vitro studies describing the adverse autocrine and paracrine effects of exposing tubular cells to high concentrations of protein [6, 7]. It is worth pointing out, however, that the majority of these in vitro studies employ high concentrations of bovine serum albumin, but diseases in man in which there is a high filtered albumin load—such as occurs in minimal change disease—do not progress, suggesting that albumin *per se* is unlikely to be a pathological agent. The corollary to the proteinuria hypothesis is that anything that reduces proteinuria should abrogate the scarring process. Used as strong supporting evidence for this argument is the observation in both diabetic and nondiabetic renal disease that angiotensin-converting enzyme (ACE) inhibitors, which reduce proteinuria more than do other antihypertensives, also slow disease progression more effectively. Thus, the association between the two is be-

ing touted as a causal relationship—if the proteinuria can be limited the damage will be limited. Is this argument fully supported by current information?

At this point, it worth considering what criteria should apply if the hypothesis that proteinuria causes progressive renal scarring is broadly applicable. The following points are relevant: (1) Non-proteinuric (or minimally proteinuric) renal diseases should not be progressive; (2) whenever there is heavy proteinuria, there should be an increased tendency for loss of renal function to ensue; (3) reduction in proteinuria, by whatever means, should correlate with slowing of disease progression; and (4) to establish with certainty that a "renoprotective" agent exerts its effect uniquely by reducing the degree of proteinuria, such an agent should be shown to have no other renoprotective effects. Of these points, only the third point appears to be emerging as fact. Studies in proteinuric, nondiabetic nephropathy, comparing the ACE inhibitor ramipril with other antihypertensive agents that achieve a comparable level of blood pressure control, have shown that the ACE inhibitor not only reduces proteinuria to a greater extent than do other antihypertensives, but that it also slows the progression of renal disease while reducing the level of proteinuria [8].

What about common renal diseases that do not fit into this mold and that do not support points 1 and 2 above? First, there is the example of hypertensive nephrosclerosis, a common cause of end-stage renal disease (particularly in the Afro-Caribbean population in the USA) in which proteinuria is not a prominent feature. Conversely, in minimal change disease, a heavy, often-relapsing proteinuric state in children, there does not appear to be convincing evidence that renal scarring occurs.

An important issue is whether ACE inhibitors are renoprotective exclusively via their antihypertensive and antiproteinuric actions (see point 4 above). Recent information suggests that these agents may act by mechanisms in addition to their blood pressure lowering and antiproteinuric effects (abstract; Jafar et al, *J Am Soc Nephrol* 10:77A, 1999). Based on a statistical analysis of the interaction between urinary protein excretion and ACE inhibitors, the treatment effect of ACE inhibitors appears to be significantly beneficial above a urinary protein excretion of around 750 mg/day (Jafar TH, personal communication). Thus, even when proteinuria is very limited, ACE inhibitors appear to be renoprotective. It could be argued that a minimal degree of proteinuria is enough to cause renal scarring and reducing it below this level is sufficient to confer protection. This, however, seems to be a highly unlikely scenario.

To gain further insight into whether protection by ACE inhibitors occurs in the absence of proteinuria, we surveyed the literature looking for evidence to show that ACE inhibitors are more renoprotective than other antihypertensive regimens in patients with essential hypertension with microalbuminuria (30–300 ng/day). We were able to identify only 3 studies of at least 12 months duration in which the total number of patients studied was small (Table 1) [9–11]. A meta-analysis of these limited studies shows no significant difference between ACE inhibitors and other antihypertensives in terms of an effect on GFR (end point compared to baseline) although ACE inhibitors clearly reduce proteinuria (albuminuria) to a greater extent than other antihypertensive agents. It is evident that the duration of the studies of such patients, in which the rate of progression of renal disease is very slow, requires an extended follow-up period to assess whether ACE inhibitors have any advantage in the setting of minimal proteinuria, such studies are currently lacking in essential hypertension. Hopefully, the final analysis of the study by Jafar et al, alluded to above, will throw further light on this issue.

THE CHRONIC HYPOXIA HYPOTHESIS

Could there be an alternative mechanism of progression which is common to many forms of primary and secondary glomerular disease, and could ACE inhibitors work via such a mechanism? We have proposed a mechanism that we refer to as the "chronic hypoxia hypothesis" [12]. This hypothesis states that chronic oxygen deprivation to the tubulointerstitial compartment is the underlying reason for the scarring process and that the basis for this is compromise of the postglomerular capillary circulation.

The argument runs as follows: In most glomerular diseases, regardless of their cause, there is heterogeneous involvement of the glomeruli such that some glomeruli are very inflamed with substantial compromise of glomerular capillary patency while other glomeruli escape injury, at least initially, and adapt by increasing the filtration rates by vasodilating the afferent and efferent arterioles so as to improve glomerular plasma flow. Two capillary "circulations" (actually a range) coexist beyond the glomeruli. In one of these, due to the very low flow through inflamed glomeruli, blood flow and oxygen delivery into the interstitial capillary network is substantially reduced. In these regions, which show a patchy distribution, the balance between oxygen supply and demand would lead to a reduced local oxygen tension, which would be chronic and which would impact on the tubulointerstitial compartment. This "ischemia" could well be the initiating cause of renin generation and angiotensin II-dependent hypertension. The second "circulation" comprises a high-flow peritubular capillary system emanating from vasodilated glomeruli. In the vasodilated glomeruli which escape initial injury, the luck does not hold out for long. The vasodilated state, which allows for their hyperfiltration, also exposes the postglomerular capillaries to an elevated pressure (if systemic hyperten-

Table 1. Studies of essential hypertension with minimal proteinuria

Author [Ref]	Total # patients	Age *years*	Duration of study *months*	Agent	Albuminuria, *mg/day* Baseline	Albuminuria, *mg/day* Endpoint	GFR, *mL/min* Baseline	GFR, *mL/min* Endpoint
ACE inhibitors								
De Venuto [9]	34	20–60	12	Captopril	6.8 ± 1.2	5.9 ± 1.9	85 ± 5	92 ± 5
Ruilope [10]	40	31–63	12	Quinapril	68 ± 16	47 ± 14	110 ± 10	93 ± 6
Bigazzi [12]	40	54 ± 5	12	Enalapril	77 ± 10	24 ± 5	111 ± 7	102 ± 13
Other antihypertensive agents*								
De Venuto [9]				Beta-blocker	7.9 ± 1.4	7.8 ± 2.7	94 ± 8	92 ± 8
Ruilope [10]				"Standard"	52 ± 12	77 ± 25	102 ± 7	93 ± 5
Bigazzi [12]				Nicardipine	65 ± 12	53 ± 21	115 ± 9	113 ± 11

*Same studies as in A; in each study half the total number of patients were administered ACEI and half other antihypertensives

sion coexists) and to increased blood flow. The impact of hypertension would obviously be greatest on the glomerular capillaries where there is only the afferent arteriole to protect them but the hydrostatic pressure effects must also be transmitted to the postglomerular circulation, distal to the efferent arteriole. This is normally a very low-pressure circulation and in this subcompartment of the interstitium, high flows and pressures are likely to have an effect on the endothelium of the interstitial capillaries such that endothelial swelling and functional changes start to infringe on flow and oxygen delivery. The net result would be progressive hypoxia of the interstitium.

Can hypoxia *per se* induce a fibrogenic response? Hypoxia is a potent regulator of gene expression altering expression (both negatively and positively) of a broad spectrum of molecules including growth factors, hormones, vasoactive compounds and enzymes involved in intermediary metabolism [13–16]. In a series of in vitro studies we have shown that hypoxia (1% O_2) is a profibrogenic stimulus for tubular epithelial cells, interstitial fibroblasts and renal microvascular endothelial cells (Table 2) [17; Norman et al, manuscript submitted for publication]. In response to a low pO_2, there is accumulation of extracellular matrix (ECM) proteins as a result of both increased matrix synthesis and decreased matrix degradation mediated by decreased expression of the matrix-degrading enzymes, the matrix metalloproteinases (MMPs) [19, 20], and increased production of the endogenous inhibitors of these enzymes, the tissue inhibitors of metalloproteinases (TIMPs) [21–23]. The mechanisms by which hypoxia elicits the changes in ECM metabolism in renal cells appear to involve both transcriptional and post-transcriptional events [17; Norman et al, manuscript submitted for publication]. Transcriptional regulation of genes associated with fibrogenesis has been shown to occur via both hypoxia-inducible factor-1 (HIF-1)-dependent [17; Norman et al, manuscript submitted for publication], and HIF-1-independent mechanisms (abstract; Orphanides et al, *J Am Soc Nephrol* 10:578A, 1999). Increased expression of TIMP-1 requires the activation of HIF-1, whereas up-regulation of collagen-I gene transcription is independent of this factor, suggesting that hypoxia activates multiple regulatory pathways within a single cell type.

Much has been written about the causal role of the cytokine transforming growth factor-β1 (TGF-β1) in the pathogenesis of fibrosis [23, 24]. This is merely one example of a cytokine that mediates the process of scarring once it is up-regulated. However, something must initially stimulate the growth factor and maintain it as an active participant. The same would apply to other cytokines, chemokines and infiltrating cells. The question is not whether these play a role in the fibrotic processes—they surely do—but rather, what is it that maintains the elevated levels? The "causes" of the progressive fibrosis act through these "mediators" and include proteinuria, uncontrolled hypertension and chronic hypoxia. Hypoxia is known to induce a wide variety of growth factors [13, 14], including many of those implicated in the pathogenesis of progressive renal disease, most notably TGF-β1 and platelet-derived growth factor (PDGF) [13, 23], but the up-regulation of vascular endothelial growth factor (VEGF) by low oxygen [25] may have important repercussions on microvascular endothelial cell function. Increased expression of VEGF has been reported in diseased kidneys [26] although the functional repercussions of the increase has not been established. Persistent and progressive hypoxia within the tubulointerstitium could induce and maintain increased growth factor expression in this tissue compartment.

There are many causes of scars in the kidney which are self-limiting and which may have no tendency to progress. These could occur, for example, following localized inflammation or reflux. Most chronic renal diseases, however, are associated with progressive scarring. Histologic analysis of chronically scarred kidneys reveals that much of the microvasculature is missing from the fibrotic bands of collagen and ECM [27]. Thus, whatever caused the scar in the first place, leaves the kidney with regions of hypovascularity. The cells lying around the edges of such areas would sense relative hypoxia. They, in turn, would mount a fibrogenic response which would

Table 2. Effects of hypoxia (1% O_2, 24–48 hours) on human renal cells in vitro

	PTE	CF
Viability (LDH release)	↔	↔
GAPDH mRNA expression	↑	↑
Proliferation (^{3}H-thymidine incorporation)	↔	↑
Protein synthesis (^{3}H-phenylalanine incorporation)	↓	ND
SMA expression	ND	↑
Collagen mRNA:		
Collagen I	↑	↑
Collagen III	ND	↑
Collagen IV	↔	ND
Collagen production	↑	↑
MMP mRNA:		
MMP-1	ND	↓
MMP-2	↔	ND
MMP-9	↔	ND
MMP protein:		
MMP-1	ND	↑
MMP-2	↓	↔
MMP-9	↓	↔
TIMP mRNA:		
TIMP-1	↔	↑
TIMP-2	↓	↔
TIMP-3	ND	↑
TIMP-1 protein	↑	↑
Growth factors:		
TGF-β1 mRNA	ND	↑
TGF-β1 protein	↑	↑
PDGF	↑	↑
VEGF	↑	ND
ET-1	↑	ND
Integrin expression	↑	↑
Transcription factors		
HIF-1α mRNA	↑	↑
HIF-1α protein	↑	↑

Abbreviations and symbols are: ↑: Increased expression; ↓: Decreased expression; ↔: No change in expression; CF: Cortical interstitial fibroblasts; ET-1: Endothelin-1; HIF-1α: Hypoxia-induced factor-1, alpha sub-unit; MMP: Matrix metalloproteinase; ND: Not tested; PDGF: Platelet-derived growth factor; PTE: Proximal tubular epithelial cells; SMA: α-smooth muscle actin; TGF-β1: Transforming growth factor-β1; TIMP: Tissue inhibitor of matrix metalloproteinase; VEGF: Vascular endothelial cell growth factor.

lead to the obliteration of more capillaries, thereby setting up a vicious cycle.

We would argue that, provided that there is a critical level of local vascular obliteration associated with the early phase of the disease, the surrounding cells will respond to the hypoxia by expanding the scar, which, for reasons given above, will progress continuously.

In the kidney, the loss of the microvasculature is particularly interesting in that the vessels that are obliterated are the outflow tracts of the glomeruli, i.e., the peritubular capillary network [27]. This explains why glomerular filtration would decline and glomerular drop-out would occur as tubulointerstitial fibrosis gets worse.

COULD "RENOPROTECTIVE" PHARMACOLOGICAL AGENTS ACT VIA IMPROVED TISSUE OXYGENATION?

As outlined above, claims for the efficacy of ACE inhibitors argue that the reason that they have an advantage is that they reduce proteinuria. However, it is possible that these renoprotective agents act via other, as yet unidentified, mechanisms. We therefore asked whether ACE inhibitors could protect the kidney by improving interstitial oxygen delivery. Theoretically, they should be able to do this because they dilate the efferent arterioles, reduce renal vascular resistance and they should improve microvascular flow in the interstitium. To address this, we recently described an in vivo model in which the phosphorescence of an injected protoporphyrin is inversely related to microvascular pO_2 (abstract; Norman et al, *J Am Soc Nephrol* 10:666A, 1999). This phosphorescence can be measured noninvasively on the surface of an exposed rat kidney. Preliminary findings suggest that an ACE inhibitor prevents the slow decline in pO_2 within the kidney that occurs over a 3-hour period of observation, and that once such a decline has begun, it can be arrested and pO_2 increased by bolus administration of an ACE inhibitor. These findings suggest a new renoprotective mechanism of action for ACE inhibitors which hinges on improvement of interstitial capillary pO_2. Whether this holds up for chronic administration remains to be seen.

UNPROVEN ELEMENTS OF THE CHRONIC HYPOXIA HYPOTHESIS

Our hypothesis requires that evidence of in vivo hypoxia of the kidney should occur at an early stage of the progressive kidney disease (prior to microvascular obliteration) due to decreased peritubular capillary flow in some regions and to the endothelial responses to increased pressures and flow in others. Thus, evidence of decreased pO_2 should be demonstrable prior to histologic evidence of scarring in models of progressive disease.

It also requires that hypoxia persists throughout the course of the disease which includes the phase of microvascular obliteration. The ability to detect this in vivo is now feasible since, even where there is patchy scarring, reduced net perfusion of a volume of renal tissue can be sensed. It will thus be possible to test the hypothesis fully and to determine whether initiation of therapy with ACE inhibitors prior to the onset of the disease can delay its onset by improving renal interstitial oxygenation at the earliest stage of the disease.

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REFERENCES

1. Fine LG: Preventing the progression of human renal disease. Have rational therapeutic principles emerged? *Kidney Int* 33:116–128, 1988
2. Jacobson H: Chronic renal failure: Pathophysiology. *Lancet* 338:419–427, 1991
3. Bohle A, Wehrmann M, Bogenschütz O, Batz C, Vogl W, Schmitt H, Müller CA, Müller GA: The long term prognosis of primary glomerulonephritides. A morphological and clinical analysis of 1747 cases. *Pathol Res Pract* 188:908–924, 1992
4. Anderson S, Brenner BM: Progressive renal disease: a disorder of adaptation. *Q J Med* 70:185–189, 1989
5. Remuzzi G, Ruggenti P, Benigni A: Understanding the nature of renal disease progression. *Kidney Int* 51:12–15, 1997
6. Burton CJ, Walls J: Interstitial inflammation and scarring: Messages from the proximal tubular cells. *Nephrol Dial Transpl* 11:1505–1523, 1996
7. Zoja C, Benigni A, Remuzzi G: Protein overload activates proximal tubular cells to release vasoactive and inflammatory mediators. *Exp Nephrol* 7:420–428, 1999
8. The GISEN Group (Gruppo Italiano di Studi Epidemedilogici in Nephrologia): Randomised placebo-controlled trail of the effect of ramipril on decline in glomerular filtration rate and risk of terminal renal failure in proteinuric, non-diabetic nephropathy. *Lancet* 349:1857–1863, 1997
9. De Venuto G, Andreotti C, Mattarei M, Pegoretti G: Long-term Captopril therapy at low doses reduces albumin excretion in patients with essential hypertension and no sign of renal impairment. *J Hypertens* 3 (Suppl. 2):S143–S145, 1985
10. Ruilope LM, Alcazar JM, Hernandez E, Praga M, Lahera V, Rodicio JL: Long-term influences of anithypertensive therapy on microalbuminuria in essential hypertension. *Kidney Int* 45 (Suppl. 45):S171–S173, 1994
11. Bigazzi R, Bianchi S, Baldari D, Sgherri G, Baldari G, Campese VM: Long-term effects of a converting enzyme inhibitor and a calcium channel blocker on urinary albumin excretion in patients with essential hypertension. *Am J Hypertens* 6:108–113, 1992
12. Fine LG, Orphanides C, Norman JT: Progressive renal disease: The chronic hypoxia hypothesis. *Kidney Int* 53 (Suppl. 65):S74–S78, 1998
13. Bauer C, Kurtz A (editors): Forefronts in Nephrology: Oxygen sensing on the cellular and molecular levels. *Kidney Int* 51:371–608, 1997
14. Bunn HF, Poynton R: Oxygen sensing and molecular adaptation to hypoxia. *Physiol Rev* 76:839–885, 1996
15. Shih S-C, Claffey KP: Hypoxia regulation of gene expression in mammalian cells. *Int J Exp Pathol* 70:347–357, 1998
16. Ratcliffe PJ, O'Rourke JF, Maxwell PH, Pugh CW: Oxygen sensing, hypoxia-inducible factor 1 and the regulation of gene expression. *J Exp Biol* 201:1153–1162, 1998
17. Orphanides C, Fine LG, Norman JT: Hyoxia stimulates proximal tubular cell matrix production via a TGF-β1 independent mechanism. *Kidney Int* 52:637–647, 1997
18. Deleted in proof.
19. Birkedal-Hansen H, Moore WGI, Bodden MK, Windsor LJ, Birkedal-Hansen B, Decarlo A, Engler JA: Matrix metalloproteinases: a review. *Crit Rev Oral Biol Med* 4:197–250, 1993
20. Stetler-Stevenson WG: Dynamics of matrix turnover during pathologic remodeling of the extracellular matrix. *Am J Pathol* 148:1345–1350, 1996
21. Denhardt DT, Feng B, Edwards DR, Cocuzzi ET, Malyankar UM: Tissue inhibitor of metalloproteinases (TIMP aka ERP): Structure, control of expression and biological functions. *Pharmacol Ther* 59:329–341, 1993
22. Gomez DE, Alonso DF, Yoshiji H, Thorgiersson UP: Tissue inhibitors of metalloproteinases: structure, regulation and biological functions. *Eur J Cell Biol* 74:111–122, 1997
23. Eddy A: Molecular insights into renal fibrosis. *J Am Soc Nephrol* 7:2495–2508, 1996
24. Border W, Noble N: TGF-β in kidney fibrosis: a target for gene therapy. *Kidney Int* 51:1388–1396, 1997
25. Enholm B, Paavonen K, Risitmaki A, Kumar V, Gunji Y, Klefstrom J, Kivinen L, Laiho M, Olofsson B, Joukov V, Eriksson U, Alitalo K: Comparison of VEGF, VEGF-B, VEGF-C and Ang-1 regulation by serum, growth factors, oncoproteins and hypoxia. *Oncogene* 14:2475–2483, 1997
26. Gröne H-J, Simon M, Gröne EF: Expression of vascular endothelial growth factor in renal vascular disease and renal transplants. *J Pathol* 177:259–267, 1995
27. Bohle A, Gise HV, Mackensen-Haen S, Stark-Jakob B: The obliteration of the post-glomerular capillaries and its influence upon the function of both glomeruli and tubuli. *Klin Wochenschr* 59:1043–1051, 1981